Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendments, the specification has been amended to correct the clerical errors described below.

The first two paragraphs in the "BRIEF DESCRIPTION OF THE FIGURES" (paragraphs beginning on line 8 of page 6) have been amended to cancel reference to "Working Cell Banks" and instead refer to "Primary" and "Master" cell banks. Support for this amendment can be found in the specification as originally filed. For example, a description of "Primary Cell Bank" can be found in the specification in Figure 3 (data point at about 10 days in culture); and at, page 6, lines 14-16 ("Figure 3 illustrates the yield (Cell Number) of cells that co-express CD49c and CD90 cells during *ex vivo* expansion of a Primary Cell Bank of colony forming units (CFUs) derived from human bone marrow aspirates"). Similarly, "the Primary Cell Bank" is described in Example 3 as cells which have initially been grown for 7-10 days in culture.[1] Additionally, Example 1 describes these initially cultured cells (*i.e.*, cultured *in vitro* for 7-10 days) in Figure 1A as "approximately 70% CD49c positive" (page 27, lines 1-4). Example 1 also explains, "[t]he majority of cells that did not express CD49c were positive for expression of hematopoetic/myeloid lineage marker CD45 (Figure 1A, LR quadrant)..." (page 27, lines 4-5). Hence, the cells analyzed in Figure 1A for CD49c/CD45 expression correspond to "Primary Cell Bank" cells.

Likewise, Example 2 describes these initially cultured cells (*i.e.*, cultured *in vitro* for 7-10 days) in Figure 2A as "approximately 50%" positive for expression of CD49c (page 27, line 27 to page 28, line 1). Example 2 also explains, "[t]he majority of cells that did not express CD49c were positive for expression of hematopoetic/myeloid lineage

[1] "After 7-10 days in culture, the CFUs generated using the methods described in Example 1 were removed from the T75 flasks with a 0.25% trypsin/1 mM EDTA solution (Life Technologies). After 10 minutes at 37°C., the trypsin was inactivated with 10 mL of complete medium. The cells were washed once with HBSS and re-suspended in Glycerol Cell Freezing Medium® (Sigma Chemical Co.). Aliquots (referred to herein as "the Primary Cell Bank"..." *See*, specification, page 28, lines 7-15.

marker CD45 (Figure 2A, LR quadrant)..." (page 28, lines 1-3). Hence, the cells analyzed in Figure 2A for CD49c/CD45 expression correspond to "Primary Cell Bank" cells.

A typographical error wherein CD49c was incorrectly typed as "CD40c" has been corrected (paragraph beginning on line 12 of page 15). This clerical error would have been apparent to those of ordinary skill in the art because the specification throughout refers to CD49c and nowhere else does it refer to "CD40c." Furthermore, Applicants are not aware of the existence of any naturally occurring "CD40c" cell surface proteins. No new matter has been added by this amendment.

The paragraph beginning on line 18 of page 26 and bridging to page 27, line 8 of the specification has been amended to delete the phrase "More than 94% of the adherent population was CD90 and CD49c positive (Figure 1B)" from Example 1.

This same phrase has been moved to Example 3, in the paragraph beginning on line 22 of page 28. Support for this amendment can be found in the specification as originally filed. For example, the paragraph beginning on line 18 of page 26 and bridging to page 27, line 8 of the specification (Example 3) states "The purity of the cells (percentage of cells which co-express CD49c/CD90) in the Master Cell Bank was determined by flow cytometry using the same method as above." Furthermore, in Figure 1, panel 1B is the only panel which shows simultaneous flow cytometry analysis of CD49c (y-axis) and CD90 (x-axis). Hence, the cells analyzed in Figure 1B for CD49c/CD90 expression correspond to "Master Cell Bank" cells as described in Example 3. *See also,* Figure 3 ("Master Cell Bank" cells at about 20 days in culture). This same paragraph in Example 3 has also been amended to delete "CD90" and replace this term with "CD49c" in reference to Figure 1C. Support for this amendment is supported by Figure 1C as originally filed, which shows flow cytometry analysis of CD49c expression along the y-axis.

The paragraph in Example 1, beginning on line 3 of page 26 has been amended to delete "FICOLL®" preceding the phrase "density gradient separation." As described in Example 2 ("Isolation of a Adherent CFUs from Bone Marrow Aspirates Following Density Separation"), the density gradient separation procedure utilized "HISTOPAQUE 1.119 (Sigma, St. Louis, MO)" and reference to FICOLL in Example 1 was a minor

clerical error. Support for this amendment can found in Example 2 (pages 27-28) which provides a detailed description for the isolation of adherent cells from bone marrow aspirates via density separation.

The paragraph in Example 2, beginning on line 11 page 27 has also been amended to capitalize all letters in the trademarked term "HISTOPAQUE® 1.119" in compliance with guidelines presented in M.P.E.P. § 608.01 (v).

The paragraph beginning beginning on line 27 of page 27 and bridging to line 6 of page 28 of the specification has been amended to delete the phrase "More than 91% of the adherent population was CD90 and CD49c positive (Figure 2B)" from Example 2.

This same phrase has been moved to Example 3, in the paragraph beginning on line 1 of page 29 (the words "the" and "and" were also inserted to make the amended sentence grammatically correct). Support for this amendment can be found in the specification as originally filed. For example, in Example 3, the paragraph beginning on line 18 of page 26 and bridging to page 27, line 8 of the specification states "The purity of the cells (percentage of cells which co-express CD49c/CD90) in the Master Cell Bank was determined by flow cytometry using the same method as above." Furthermore, in Figure 2, panel 2B is the only panel which shows simultaneous flow cytometry analysis of CD49c (y-axis) and CD90 (x-axis). Hence, the cells analyzed in Figure 2B for CD49c/CD90 expression correspond to "Master Cell Bank" cells as described in Example 3. *See also*, Figure 3 ("Master Cell Bank" cells at about 20 days in culture). This same paragraph in Example 3 has also been amended to delete "CD90" and replace this term with "CD49c" in reference to Figure 2C. Support for this amendment is supported by Figure 2C as originally filed, which shows flow cytometry analysis of CD49c expression along the y-axis.

No new matter is believed to have been added by these clarifying amendments.

Claims 14, 21, 25 and 26 are pending in the application, with claim 14 being the sole independent claim. Claims 1-13, 15-18, 22-24, and 27-96 were previously canceled without prejudice to or disclaimer of the subject matter therein. Claims 19 and 20 have been canceled herein without prejudice to or disclaimer of the subject matter therein. Independent claim 14 has been amended to, once again, include the parameter "greater

than about 91% of " (as suggested by the Examiner and as discussed further below). Hence, the amendments are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Claim Rejections Under 35 U.S.C. § 112

The Examiner has currently rejected claims 14, 19-21 & 25-26 under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. *See*, Paper No. 20070930, page 2. In particular, the Examiner alleges:

> Applicant has amended the claims to remove the limitation "greater than 91%" and by doing so is claiming a pure composition of the claimed cell phenotype. It is not clear that applicant has support in the instant specification for a pure population of the claimed phenotype therefore the amendment is considered to be new matter.

Id., penultimate paragraph.

Applicants respectfully disagree with this rejection. As an initial matter, claims 19 and 20 have been canceled herein. Therefore, the rejection with respect to these claims has been rendered moot.

Applicants also note that the specification as originally filed provides sufficient written description support for "a pure population of the claimed phenotype." For example, the specification at page 28, Example 2 (last sentence), teaches "More than 91% of the adherent population was CD90 and CD49c positive (Figure 2B)." Hence, *more than 91%* includes 100% or, in other words, "a pure population." Despite the presence of such sufficient written description support, Applicants have nonetheless amended claim 14 (and thereby dependent claims 21 and 24-25) to once again include the term "greater than about 91%." Accordingly, the rejection of pending claims 14, 21 and 25-26 under 35 U.S.C. § 112, first paragraph, has been obviated and withdrawal of the rejection is respectfully requested.

The Examiner has rejected claims 14, 19-21 & 25-26 under 35 U.S.C. § 112, first paragraph, as allegedly containing new matter. *See*, Paper No. 20070930, page 2, last paragraph. In particular, the Examiner alleges:

> Further, the limitation "after 30 doublings" is considered to be new matter. Applicant points to support in the specification for this limitation however at the point of reference, the specification says after 50 doublings and there is no apparent support for the smaller range.

Id.

Applicants respectfully disagree with this rejection. As an initial matter, claims 19 and 20 have been canceled herein. Therefore, the rejection with respect to these claims has been rendered moot.

Applicants note that the specification at page 29, lines 25 to 27 was previously pointed to in support of the claim amendment "after 30 cell doublings." More particularly, the relevant part of the specification at this point reads "The doubling rate (# days in culture x 24/doublings) was 30 hrs and remained constant for at least 50 doublings. *Even after 30 doublings*, the population uniformly retained..." (emphasis added)[2]. Additionally, Applicants note that Figures 3 and 4 also provide explicit support for the claim parameter "after 30 cell doublings" as these figures clearly show that the cell population doubling rate is maintained both before and after 30 cell population doublings. Hence, Applicants respectfully submit that the specification provides sufficient written description support for "the smaller range" of 30 cell doublings. Accordingly, the rejection of pending claims 14, 21 and 25-26 under 35 U.S.C. § 112, first paragraph, has been obviated and withdrawal of the rejection is respectfully requested.

Prior Art

Applicants thank the Examiner for consideration of Applicants' previously submitted evidence and arguments and, subsequent thereto, for withdrawal of the

[2] The complete sentences at this portion of the specification (*i.e.*, bridging over to page 30, lines 1-2) read: "The doubling rate (# days in culture x 24/doublings) was 30 hrs and remained constant for at least 50 doublings. Even after 30 doublings, the population uniformly retained the characteristic morphology of small, dividing cells without apparent evidence of the enlarged, flat morphology of aged or terminally-differentiated cells."

Examiner's previous rejection based on Furcht *et al.* (U.S. Patent No. 7,015,037). *See*, Paper No. 20070930, page 3, first paragraph.

I. Claim Rejections Under 35 U.S.C. § 102

The Examiner has rejected claims 14, 19-21 & 25-26 as allegedly anticipated under 35 U.S.C. § 102 (b). In particular, the Examiner alleges:

> Claims 14, 19-21 & 25-26 are rejected under 35 U.S.C. 102(b) as being anticipated by Haynesworth *et al.* (1998, U.S. Patent 5,733,542) taken in light of Pittenger *et al.* (1999, Science 284: 143-147), Woodbury *et al.* (2000, Journal of Neuroscience Research 61: 364-370), and Lee *et al.* (2000, Hepatology 40: 1275-1284 [*sic*[3]]).

See, Paper No. 20070930, page 3, last two paragraphs.

As an initial matter, Applicants note that Haynesworth *et al.*, U.S. Patent 5,733,542 was previously cited to the Examiner as reference "AF2" in Applicants' Second Supplemental Information Disclosure Statement *submitted on May 17, 2002.* Furthermore, this reference was signed as considered by the Examiner *on July 16, 2003*!

In the present office action, the Examiner now asserts that "Haynesworth *et al.* teach a population of mesenchymal stem cells (MSCs) isolated from human adult bone marrow (Example 1; column 3, line 18, through column 4, line 51)." The Examiner then explains reference to the remaining cited publications by stating:

> Pittenger *et al.* is cited as evidence that the MSC's of Haynesworth *et al.* can differentiate to various mesodermal cell lineages, including bone, cartilage, and adipose...
>
> Woodbury *et al.* is cited as evidence that the MSCs of Haynesworth *et al.* can differentiate to neurons...
>
> Lee *et al.* is cited as evidence that the MSCs of Haynesworth *et al.* can differentiate to hepatocytes...

Id. at page 4, 1st through 4th paragraphs.

[3] Lee *et al.* is a 2004 publication (not a 2000 publication) and, thus, is not prior art under 35 U.S.C. § 102 (b).

The Examiner has also rejected claims 14, 19-21 & 25-26 under 35 U.S.C. § 102 (b) as allegedly anticipated by Haynesworth in view of Jiang *et al.* *See*, Paper No. 20070930, page 7, 1st paragraph. In particular, the Examiner alleges:

> Jiang *et al* is cited as evidence that Haynesworth *et al.*'s method of isolating their MSCs inherently yields a population that comprises mostly MSCs but also another rare cell type, MAPCs, that co-purify with the MSCs and that differentiate into mesodermal as well as ectodermal and endodermal cell lineages (Abstract; Figure 3). Jiang *et al.* teach that MAPCs can undergo at least about 100 population doublings (page 41, column 2, paragraph 1).

Id., at 3rd paragraph.

In attempt to justify the rejections under 35 U.S.C. § 102(b), the Examiner cites an assortment of case law and includes therewith a number of generalizations about the doctrine of inherent anticipation (*i.e., Standard Havens Prods., Inc. v. Gencor Indus., Inc.*, 953 F.2d 1360 (Fed. Cir. 1991); *Verdegaal Bros., Inc. v. Union Oil Co. of Cal*, 814 F.2d 628 (Fed. Cir. 1987); *In Re King,* 801 F.2d 1324 (Fed. Cir.1986); *Titanium Metals,* 778 F.2d 775 (Fed. Cir. 1985); *Atlas Powder Co. v. IRECO Inc.,* 190 F.3d 1342 (Fed. Cir. 1999); and, *Continental Can Co. USA, Inc. v. Monsanto Co.,* 948 F.2d 1264 (Fed. Cir. 1991). *See*, Paper No. 20070930, page 4, last paragraph to page 6, second paragraph.

Applicants are generally in agreement with these doctrinal affirmations on inherent anticipation as set forth by the Examiner in Paper No. 20070930, page 4, last paragraph to page 6, second paragraph. Applicants respectfully differ, however, in the application of these cases to the facts at hand. Thus, despite the cited case law and explanations of some aspects of the doctrine of inherent anticipation, the Examiner's ultimate conclusion that the present claims are inherently anticipated by Haynesworth (in view of Pittenger, Woodbury, Lee, and Jiang) is in error from both a legal and a biological perspective.

Applicants also note that claims 19 and 20 have been canceled herein. Therefore, the rejection with respect to these claims has been rendered moot.

A. The Examiner's conclusion that the present claims are inherently anticipated by Haynesworth (in view of Pittenger, Woodbury, Lee, and Jiang) is legally in error.

Applicants respectfully submit that the Examiner's rejection under 35 U.S.C. § 102(b) is legally incorrect and should be withdrawn because the Examiner has not established a necessary and crucial element required for proper application of a rejection based on inherent anticipation. In particular, the Examiner has not established that the missing descriptive matter is *necessarily* present in the prior art. In this regard, the Examiner's assertions that the presently claimed invention is inherently anticipated is based on mere conjecture and speculation that an isolated cell population described in Haynesworth *might possibly* contain a cell population encompassed by the present claims. In contrast to the Examiner's unsubstantiated conjecture, however, the judicially created doctrine of inherent anticipation requires that a rejection based on inherent anticipation *cannot be based on mere possibilities or probablities*; the missing matter must *necessarily* flow from the prior art.

For example, the M.P.E.P. clearly explains the requisite burden of proof that an Examiner must provide in asserting a rejection based on inherent anticipation. In particular, the M.P.E.P. explains:

> **M.P.E.P. § 2112 - Requirements of Rejection Based on Inherency; Burden of Proof**
>
> IV. EXAMINER MUST PROVIDE RATIONALE OR EVIDENCE TENDING TO SHOW INHERENCY
>
> *The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic.* In re Rijckaert, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (reversed rejection because inherency was based on what would result due to optimization of conditions, not what was necessarily present in the prior art); In re Oelrich, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981). "*To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill.* ***Inherency, however, may not be established by probabilities or possibilities.*** *The mere fact that a certain thing may result from a given set of circumstances is not sufficient.*' " *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) (citations omitted).

* * *

> In relying upon the theory of inherency, *the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art.*" *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990)

M.P.E.P. § 2112, IV (underline emphasis added; bold emphasis in original).

Applicants respectfully submit that the present rejection is based merely on conjecture and speculation by the Examiner without any extrinsic evidence, basis in fact, or technical reasoning to provided to reasonably support such assertions. For example, in asserting the present rejection the Examiner states:

- "A reconsideration of the instant application *has led the examiner to believe* that the cell population claimed are a population of MSC (mesenchymal stem cells) or mixed populations of MSCs and MAPCs."

See, Paper No. 20070930, page 3, 1st paragraph (emphasis added).

- "While the prior art does not clearly disclose all of applicant's claimed limitations, *it would appear* that the cells claimed are a population of MSC's as disclosed by Haynesworth."

Id. at page 6, last paragraph, 1st sentence (emphasis added).

- "*It is the examiner's contention* that the disclose properties [*sic*] which are not explicitly discussed in the prior art are inherent properties of the cells and cell populations disclosed in the Haynesworth reference, however *if that is not the case it is apparent* that the claimed characteristic of the cell population are a result of different culture conditions, or the ratio of MSCs to MAPC (as discussed in Jiang) *or even the ratio of MSCs to an undetermined cell cellular 'contaminant'* [*sic*] thus effecting the population as a whole.

Id. at page 9, last paragraph, 2nd sentence (emphasis added).

- "*It would appear* that any actual differences between the cell population of Haynesworth and the claimed cell populations would be a question of the concentration of cells or the purity of the population."

Id. at page 9, last paragraph, last full sentence (emphasis added).

Thus, the Examiner contends by mere speculation, and without any supporting evidence or technical reasoning, that Applicants' claims are inherently anticipated because Applicants' presently claimed cell population *might possibly* be part of a population of cells isolated by Haynesworth or *might result* from different culture conditions, or the ratio of MSCs to MAPC, or a "cellular 'contaminant.'" This is not evidence that the missing elements *necessarily* flow from the prior art and, thus, such speculation is directly contrary to law.

Moreover, if the Examiner has rendered the present rejection based on any facts within the Examiner's personal knowledge, then it is respectfully requested that the Examiner submit an affidavit setting forth the specific facts supporting the rejection so that Applicants can have opportunity to provide contradiction or explanation pursuant to 37 C.F.R. § 1.104 (d) (2).[4] Otherwise, Applicants respectfully request reconsideration and withdrawal of the rejection of pending claims 14, 21 and 25-26.

B. The Examiner's conclusion that the present claims are inherently anticipated by Haynesworth (in view of Pittenger, Woodbury, Lee, and Jiang) is also incorrect from a biological perspective.

1. The cell populations of the present invention are distinct from the MSCs of Haynesworth as evidenced by the differing expression of CD44 cell surface markers.

As described in the Declaration by Dr. Gene Kopen submitted herewith (the "Second Kopen Declaration"), the isolated MSC cell populations disclosed in Haynesworth are **CD44** *negative*. *See*, Caplan *et al.*, U.S. Patent 5,486,359 (hereinafter "Caplan '359") and Caplan *et al.*, U.S. Patents 5,811,094 (hereinafter "Caplan '094").[5] In

[4] When a rejection in an application is based on facts within the personal knowledge of an employee of the Office, the data shall be as specific as possible, and the reference must be supported, when called for by the applicant, by the affidavit of such employee, and such affidavit shall be subject to contradiction or explanation by the affidavits of the applicant and other persons. 37 C.F.R. § 1.104 (d) (2).

5 Caplan *et al.*, U.S. Patent 5,486,359 and Caplan *et al.*, U.S. Patent 5,811,094 were previously cited to the Examiner as references "AC" and "AG", respectively, in an IDS submitted to the Patent Office on Jan. 22, 2002 and again submitted to the Patent Office on Dec. 22, 2003. *See*, "Transmittal of Previously

contrast, in the Declaration by Dr. Gene Kopen previously submitted on May 18, 2007 (hereinafter the "May 2007 Declaration") it was demonstrated that the cell populations of the present invention are **CD44** *positive*.

In this regard, in the present office action the Examiner stated:

> A reconsideration of the instant application has led the Examiner to believe that *the cell population claimed are populations of MSC* (mesenchymal stem cells) or mixed populations of MSCs and MAPCs.

See, Paper No. 20070930, page 3, 1st paragraph (emphasis added). However, given that *MSCs are* **CD44** *negative*, whereas cells of the present invention are **CD44** *positive* it is an impossibility that "the cell population claimed are populations of MSC." Hence, the **CD44** *negative* MSC cell population isolated by Haynesworth is an inherently different cell population from the **CD44** *positive* cell population of the present invention.

2. Haynesworth's MSCs are obtained from a low density gradient fraction whereas Applicants' cell populations are obtained from a high density gradient fraction.

As explained in the Second Kopen Declaration, Haynesworth's gradient fractionated MSC cells are localized within a population of *low density cells* corresponding to the *platelet fraction* of bone marrow derived cells. *See*, Caplan '359, column 17, lines 7-24 (emphasis added).

In contrast, as discussed in the Second Kopen Declaration, the gradient fractionation procedure in the present application teaches that Applicants bone marrow-derived cell population is isolated from within a *high-density mononuclear cell fraction*. *See*, Specification, page 27, lines 9-19.

Furthermore, as explained in the Second Kopen Declaration (and supported by **Exhibit B)**, the different gradient fractionation mediums used by Haynesworth (70% Percoll) and that used in the present application (Histopaque 1.119®), could *not* be used to

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Filed Information Disclosure Statement and Previously Cited References," submitted by Applicants on Dec. 22, 2003.

isolate *both* the MSCs of Haynesworth *and* the isolated cell populations of the present invention. Thus, the MSCs described by Haynesworth and the isolated cell populations of the present invention are different starting populations of cells even before any subsequent seeding/plating/culturing procedures.

3. Haynesworth teaches that specific media is "critical" for isolation of MSCs.

Isolation of Haynesworth's MSC cell population is taught to require specific media which provides "*the critical step*" for isolation "*of only the mesenchymal stem cells.*" As explained in the Second Kopen Declaration (and supported by **Exhibit C**), Caplan '359 describes three specific types of media which are critical to selective adherence of Haynesworth's MSCs. *See*, Caplan '359, column 6, line 45 through column 9, line 35.

In contrast, as described in the Second Kopen Declaration, the media used to isolate and culture cell populations of the present invention is different and distinct from the three "critical" types of media described for use in isolating Haynesworth's MSCs.

Therefore, given the teaching in Caplan '359 regarding the critical nature of the defined media developed for the selective adherence and isolation of Haynesworth's MSCs, it is improbable conjecture and speculation that Applicants media might also allow adherence, isolation, and propagation of the same cell population described in Haynesworth.

4. MSCs have longer population doubling times and diminished self-renewal capacity compared to the cell populations of the present invention.

As discussed in the Second Kopen Declaration, additional characteristics of the MSCs of Haynesworth are described in a 1997 publication titled "Growth Kinetics, Self-Renewal, and the Osteogenic Potential of Purified Human Mesenchymal Stem Cells During Extensive Subcultivation and Following Cryopreservation." *See*, Bruder, Jaiswal & Haynesworth, *Jour. Cell. Biochem. 64*:278-294 (1997)(hereinafter "Bruder").[6,7] Among

[6] The methods used in obtaining the MSCs in Bruder *et al.* are the same as described in Haynesworth '542. *Compare*, Haynesworth '542, Example 1, col. 3, line 57 to col. 4, line 51 *and* Caplan '359 Example 1, col.

characteristics discussed, the MSCs of Haynesworth are descibed in Bruder as: 1) acquiring increasingly longer population doubling times with each passage in culture; and, 2) having an average self-renewal capacity of only 38 +/- 4 population doublings (with the cells, thereafter, "degenerating"). *See, for example*, Bruder at: page 278 (Abstract); page 284, left column, second paragraph; page 284, right column, last paragraph to page 285, left column, first paragraph; and, page 285, left column, last paragraph.

As discussed and demonstrated in the Second Kopen Declaration, according to Bruder the population doubling times of Haynesworth's MSCs become progressively longer with each passage in culture. Moreover, the MSC population doubling times are also intially, and subsequently, longer than 30 hours/population doubling (with doubling times ranging, at best, from ~54 to more than 100 hours/doubling). *See*, Second Kopen Declaration. Thus, the MSCs of Haynesworth are not only a different starting cell population from those disclosed in the present application, but they also have substantially different properties upon further *in vitro* cultivation compared to the presently pending claimed cell populations.

In sum, as demonstrated via the Second Kopen Declaration, the MSCs of Haynesworth differ from the cell populations of the present invention because:

1) Haynesworth's MSCs are CD44 negative, whereas cell populations of the present invention are CD44 positive;

2) Haynesworth's MSCs are obtained from a low density, platelet containing gradient fraction, whereas cells of the present invention are obtained from a high density mononuclear cell containing fraction;

17, lines 7-45 *with* Bruder, page 279, right col., last paragraph to page 280, left column, first paragraph. Applicants also note that each of the authors in Bruder are affiliated with Case Western Reserve University ("Case Western") and/or Osiris Therapeutics, Inc. ("Osiris"). Moreover, the Haynesworth '542 and Caplan '359 patents were both assigned from each of the inventors to Case Western and from Case Western to Osiris. *See*, http://assignments.uspto.gov/assignments/?db=pat

[7] Bruder *et al.*, *Jour. Cell. Biochem. 64*:278-294 (1997) was previously submitted by Applicants as "Exhibit E" along with a Reply by Applicants submitted on Dec. 22, 2003. Applicants have included a copy of Bruder *et al.* as part of the Information Disclosure Statement submitted herewith.

3) Haynesworth's three specific media formulations are described as "*critical*" to isolation "*of only the mesenchymal stem cells*", whereas a different media formulation is used to isolate cells of the present invention;

4) Haynesworth's MSCs have reduced self-renewal capacity compared to cells of the present invention; and,

5) Haynesworth's MSCs acquire increasingly longer population doubling times with each passage in culture, whereas cell populations of the present invention maintain consistent population doubling times even after 30 population doublings.

C. Claims are not anticipated in view of Pittenger.

The Examiner alleges the presently pending claims are anticipated by Haynesworth in view of Pittenger, and asserts that Pittenger is "cited as evidence that the MSC's of Haynesworth *et al.* can differentiate to various mesodermal cell lineages, including bone, cartilage, and adipose (Figure 2)." *See*, Paper No. 20070930, page 4, 2nd paragraph. Regardless of whether or not the cells of Haynesworth can differentiate into these various cell types, Applicants' cells and Haynesworth's MSCs are different cell populations (as discussed above). Therefore, Haynesworth does not anticipate in view of Pittenger (or any other reference).

Furthermore, Applicants note that Pittenger *et al.*, *Science 284*:143-147 (1999) was previously cited to the Examiner as reference "AZ2" in an IDS *submitted to the Patent Office on Jan. 22, 2002 and again submitted to the Patent Office on Dec. 22, 2003. See*, "Transmittal of Previously Filed Information Disclosure Statement and Previously Cited References," submitted by Applicants on Dec. 22, 2003. Furthermore, this reference was signed as considered by the Examiner *on Mar. 15, 2004*!

For the sake of thoroughness in the present reply, however, Applicants submit that the presently pending claims are also not anticipated in view of Pittenger, with or without Haynesworth. In particular, as discussed in the Second Kopen Declaration, Pittenger describes a population of MSCs which are **CD106** *and* **CD62L** *positive. See*, Pittenger, page 144, left column; *see also*, Supplemental Web data at

http://www.sciencemag.org/feature/data/983855.dtl (*cited in* Pittenger at page 143, right column, 1st paragraph) (previously cited to the Examiner as reference AV6 in Applicants Ninth Supplemental IDS submitted on Oct. 4, 2006).

In contrast, as discussed in the Second Kopen Declaration (and supported by **Exhibit D**), the isolated cell populations of the present invention are **CD106** and **CD62L** *negative*. Thus, the isolated cell populations of the present invention are not anticipated by Pittenger or by Haynesworth in view of Pittenger.

D. Claims are not anticipated in view of Lee.

The Examiner alleges the presently pending claims are anticipated by Haynesworth in view of Lee, and asserts that Lee is "cited as evidence that the MSCs of Haynesworth *et al.* can differentiate to hepatocytes...". *See*, Paper No. 20070930, page 4, 4th paragraph. Regardless of whether or not the cells of Lee can differentiate into hepatocytes, Applicants' cells and Lee's MSCs are different cell populations. Therefore, Haynesworth does not anticipate in view of Lee (or any other reference).

For the sake of thoroughness in the present reply, however, Applicants submit that the presently pending claims are not anticipated in view of Lee.

As an initial matter, Lee is a December 2004 publication and is, therefore, not prior art against the present application.

Second, Lee describes the surface phenotype of their MSCs as "*consistent with classical MSCs*[26]" (citing the same Pittenger referenced discussed herein). *See*, Lee, page 1282, left column, 1st paragraph. Among these surface phenotype markers, the "classical" MSCs of Lee are shown to be **CD13** *negative*. *See*, Lee, page 1278, right column ("The fibroblast-like morphology of BM-derived cells...as well as their surface phenotype (Fig. 1B), as determined by flow cytometry, were consistent with those reported in the literature for MSCs.[26[Pittenger]] These BM-derived cells were negative for CD13..."). Moreover, Lee's classical MSCs also have longer doubling times than the population doubling rate of the presently claimed cell populations. In particular, Lee states "The doubling time of these BM-derived cells was found to be between 40 to 52 hours (data not shown)." *See*, Lee at page 1278, right column, 1st paragraph.

In contrast to the MSCs described by Lee, the isolated cell populations of the present invention are **CD13** *positive*, as discussed in the Second Kopen Declaration and as shown in **Exhibit D**. Thus, the isolated cell populations of the present invention are not anticipated by Lee, or by Haynesworth in view of Lee.

E. Claims are not anticipated in view of Woodbury.

The Examiner alleges the presently pending claims are anticipated by Haynesworth in view of Woodbury, and asserts that Woodbury is "cited as evidence that the MSCs of Haynesworth *et al.* can differentiate to neurons..." *See*, Paper No. 20070930, page 4, 3rd paragraph. Regardless of whether or not the cells of Woodbury can differentiate into neurons, Applicants' cells and Woodbury's MSCs are different cell populations. Therefore, Haynesworth does not anticipate in view of Woodbury (or any other reference).

Furthermore, Applicants note that Woodbury *et al.*, *J. Neurosci. Res. 61*: 364-370 (2000) was previously cited to the Examiner as reference "AX3" in an IDS *submitted to the Patent Office on Jan. 22, 2002 and again submitted to the Patent Office on Dec. 22, 2003*. *See*, "Transmittal of Previously Filed Information Disclosure Statement and Previously Cited References," submitted by Applicants on Dec. 22, 2003. Furthermore, this reference was signed as considered by the Examiner *on Mar. 15, 2004*!

For the sake of thoroughness in the present reply, however, Applicants submit that the presently pending claims are not anticipated in view of Woodbury.

As an initial matter, the bulk of the data and discussion in Woodbury is about *rat* MSCs, not human MSCs. Indeed, Woodbury contains only one paragraph describing the results of one experiment with human MSCs. *See*, Woodbury, page 368 ("Human Stromal Cells Differentiate Into Neurons"). In this regard, Applicants note that the presently claimed *human* cell populations of the presently pending claims cannot be anticipated by the *rat* cells of Woodbury.

Furthermore, as discussed in the Second Kopen Declaration, the human MSCs described in Woodbury are the same as those described in Azizi *et al.*, "Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats -

similarities to astrocyte grafts," *Proc. Natl. Acad. Sci. USA. 95*:3908-3913 (1998)(hereinafter "Azizi").[8] However, also as discussed in the Second Kopen Declaration, the MSCs of Azizi are not the same population of cells disclosed in Applicants present specification. For example, sufficient growth and proliferation of the human MSCs in Azizi requires cell culture supplementation with PDGF-AA (which Applicants' cell populations do not require; as previously discussed in detail in the May 2007 Declaration). *See e.g.*, Azizi, page 3911, left column, first paragraph. Additionally, as discussed in the Second Kopen Declaration, the hMSCs of Azizi have a population doubling time (PD) of, at best, approximately 96 hours *with* PDGF-AA supplementation. *See*, Azizi *et al.*, p. 3909, Fig. 1. Accordingly, given the differences (discusssed herein and in the Second Kopen Declaration) between the isolated cell populations of Azizi, and therefore of Woodbury, it is clear that the isolated cell populations of the present invention are quite distinct from the "hMSCs" of Woodbury.

F. Claims are not anticipated in view of Jiang.

The Examiner stated:

> Jiang *et al* is cited as evidence that Haynesworth *et al.*'s method of isolating their MSCs inherently yields a population that comprises mostly MSCs but also another rare cell type, MAPCs, that co-purify with the MSCs and that differentiate into mesodermal as well as ectodermal and endodermal cell lineages (Abstract; Figure 3). Jiang *et al.* teach that MAPCs can undergo at least about 100 population doublings (page 41, column 2, paragraph 1).

See, Paper No. 20070930, page 7, 3rd paragraph.

As an initial matter, Jiang is a July 2002 publication[9] and is, therefore, not prior art against the present application.

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8 Azizi *et al.*, *Proc. Natl. Acad. Sci. USA. 95*:3908-3913 (1998) was previously cited to the Examiner as reference "AS" in an IDS submitted to the Patent Office on Jan. 22, 2002 and again submitted to the Patent Office on Dec. 22, 2003. *See*, "Transmittal of Previously Filed Information Disclosure Statement and Previously Cited References," submitted by Applicants on Dec. 22, 2003.

9 Jiang *et al., "Pluripotency of mesenchymal stem cells derived from adult marrow," Nature 418:41-49 (July 2002).*

Second, regardless of whether or not the cells of Jiang differentiate into various cell lineages and can undergo 100 population doublings, Applicants' cells and Jiang's cells are different cell populations. Thus, Haynesworth does not anticipate in view of Jiang (or any other reference).

Third, Jiang describes *mouse* bone marrow-derived cells whereas the present claims are drawn to *human* cells. Hence, Jiang cannot anticipate the currently pending claims (drawn to *human* cells).

Fourth, the mouse MAPCs disclosed in Jiang are described as **CD44** and **MHC Class I** *negative*. *See e.g.*, Jiang, page 41, right column ("The phenotype of cultured mMAPCs is CD34, *CD44*, CD45, c-Kit, *and major histocompatibility complex (MHC) class I and II negative*..." (emphasis added)); *see also*, Fig 1; *and* Jiang, Y., *et al.*, Fig. 1, "*Corrigendum*: Pluripotency of mesenchymal stem cells derived from adult marrow," *Nature* 447:879-880, Nature Publishing Company (June 2007). In contrast, the Applicants previously demonstrated that the isolated cell populations of the present invention are **CD44** *and* **MHC Class I** *positive*. *See*, May 2007 Declaration. Hence, Jiang's isolated MAPCs and the isolated cell populations of the present invention are different cell populations.

Fifth, Applicants note that the credibility of the Jiang publication is currently considered with substantial skepticism by the scientific community. In fact, Jiang was previously cited to the Examiner by the Applicants as part of a discussion regarding the lack of credibility, and reproducibility, with which the scientific community currently views the claimed plasticity of cells in U.S. Patent No. 7,015,037 (Furcht *et al.*). In this regard, Applicants previously noted:

> ...the scientific community has raised serious questions with respect to the underlying data and validity of attributes alleged to be possessed by the "multipotent" bone marrow cells described in U.S. Patent No. 7,015,037 (the "Furcht patent"; currently licensed to Athersys Inc.). In February of 2007 several news articles publicly raised questions about data published by one of the co-inventors and lead investigators (Catherine Verfaillie) responsible for developing the bone marrow cells claimed in the Furcht patent. Upon request of the Examiner, copies of these publications are included herewith.
>
> - NewScientist.com, "Flawed stem cell data withdrawn" (Feb. 15, 2007)

(http://www.newscientist.com/article.ns?id=mg19325915.200)

- DeseretNews.com, "Study on uses for adult stem cells was flawed, panel says" (Feb. 25, 2007) (http://www.deseretnews.com/dn/view/1,1249,660198505,00.html)

- NYTimes.com, "Panel Finds Flawed Data in a Major Stem Cell Report" (Feb. 25, 2007) (http://www.nytimes.com/2007/02/28/science/28stem.html?ex=1174708800&en=84aaf65fc6e7ec6e&ei=5070)

- NewScientist.com, "Fresh questions on stem cell findings" (Mar. 22, 2007) (http://www.newscientist.com/article.ns?id=mg19325964.600).

> In particular, it has been reported that a key paper published in Nature [***Jiang***] (vol. 418, p. 41 (2002)) by Catherine Verfaillie and others has now been acknowledged by Verfaillie to have flawed data that should not be relied upon. This paper was initially considered by the scientific community to represent a seminal breakthrough in adult stem cell research because the isolated bone marrow cells were reported to be capable of differentiating into a surprisingly vast number of cell types.

See, Amendment and Reply, submitted May 18, 2007, pages 22-24.

Moreover, since Applicants' last reply, the journal Nature has published a Correction and Amendment with respect to errors present in the original Jiang publication. *See*, Jiang, Y., *et al.*, "*Corrigendum*: Pluripotency of mesenchymal stem cells derived from adult marrow," *Nature 447*:879-880, Nature Publishing Group (June 2007) (submitted with the Eleventh Supplemental Information Disclosure Statement on July 9, 2007). In particular, this *Corrigendum* states, "The plots for these antigens (CD19, CD34, Sca-1, Thy-1 **[a.k.a., CD90]**, and MHC-II) in the original Fig. 1b should therefore not be relied upon as an accurate representation of MAPC surface marker profiles... A corrected version of Fig. 1b is now provided as Fig. 1 below." Perhaps most informative in this regard is that the Thy-1 FACS expression plot (*i.e.*, CD90 expression data) in Fig. 1 is shifted one entire log (10-fold) to the left indicating the isolated mouse MAPCs appear negative for CD90 expression (although the *Corrigendum* authors prefer to label this data as "DIM" for Thy-1 (CD90) expression). *Compare*, *Corrigendum*, Fig. 1 "Thy-1" FACS plot *with* Jiang, Fig. 1b "Thy-1" FACS plot.

Sixth, the human MAPCs corresponding to murine MAPCs as referenced in Jiang,[10] are the same human MAPCs described in Furcht *et al.* (U.S. Patent No. 7,015,037). This is evident because Jiang describes isolating murine MAPCs via "methods identical to those used for human (h)MAPCs" as described in Reyes, *et al.*, *Blood* 98:2615-2625 (2001).[11] Moreover, Reyes *et al.* is the original journal article disclosing the hMAPCs described in Furcht *et al.* *Compare*, Furcht *et al.* *versus* Reyes, *et al.* The connection of Jiang to Furcht *et al.* is relevant to the present discussion because Applicants already overcame rejections under 35 U.S.C. §§ 102 and 103 based on the hMAPCs of Furcht *et al.*

For the record, Applicants note that a March 2007 publication by *New Scientist*[12] also recognized the connection between Reyes *et al.* and and Furcht *et al.* In this regard, *New Scientist* commented:

> Now New Scientist has examined a US patent (number 7015037) granted in 2006 that covers the isolation and use of MAPCs...
>
> Within the patent are three images that appear to be duplicated from another paper from Verfaillie's group, published in 2001 in the journal Blood (vol 98, pp 2615-2625)[*i.e.*, Reyes *et al.*]. These images relate to experiments in which MAPCs were grown in culture dishes and made to differentiate into other cell types, such as those found in bone, cartilage, fat and the linings of blood vessels. The images document the presence of proteins specific to each type of cell being produced.
>
> The problem is that in each case the duplicated image is used in the patent to describe the production of a different protein from that described in the Blood paper.

See, Aldhous, P., and Reich, E.S., "Fresh questions on stem cell findings," http://www.newscientist.com/article.ns?id=mg19325964.600, 3 pages, New Scientist

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[10] *See*, Jiang, page 41, left column, 3rd and 4th paragraphs.

[11] *Id. 4th paragraph* ("To isolate MAPCs from murine bone marrow, we used methods identical to those used for human (h)MAPCs[28]..."). *Note:* Reference 28 is Reyes, M., *et al.*, "Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells," *Blood* 98:2615-2625 (November 2001).

[12] Aldhous, P., and Reich, E.S., "Fresh questions on stem cell findings," available online at http://www.newscientist.com/article.ns?id=mg19325964.600, 3 pages, New Scientist magazine (Mar. 2007); previously provided to the Examiner as Cite No. NPL2 in Applicants' Tenth Supplemental Information Disclosure Statement.

magazine (Mar. 2007). Indeed, the publication by Reyes *et al.* as currently available from the journal *Blood*'s website comes with the following disclaimer:

> *Notice of concern: It has come to our attention, through communication with the authors and others, that there are apparent duplications in some of the figures presented in this paper. The implications of these errors on the conclusions of this paper are currently under review by Blood and the University of Minnesota. The Journal will inform the readers of the outcome of this review upon its completion.*

Prompted by these observations, Applicants have observed that there appear to be at least *three different* data images reproduced in Furcht *et al.* and Reyes *et al.*, though each are labeled as representing different proteins in the patent versus the journal publication. In particular, compare the '037 Patent, Fig. 6, "*Collagen-II*" Western blot "Day 0, 7, 12, 15" image which appears to be the same image shown in Reyes *et al.*, Fig. 5C, as an "*Osteonectin (BON-1)*" Western blot "MPC #1, #2, #3".

Compare, the '037 Patent, Fig. 6, "*Bone Sialoprotein*" Western blot "Day 0, 7, 12, 15" image which appears to be the same image shown in Reyes *et al.*, Fig. 6B as a "*Collagen II*" Western blot.

Likewise, compare *the same* '037 Patent, Fig. 6, "*Bone Sialoprotein*" Western blot "Day 0, 7, 12, 15" image which also appears to be the same image shown in Reyes *et al.*, Fig. 5C as the "*β-actin*" control for "Bone sialoprotein", except that in Reyes *et al.* this image appears to have been rotated around its vertical axis compared to the image in the '037 Patent.

Similarly, compare the '037 Patent, Fig. 10, "*Tie*" Western blot "Day 0, 7, 12, 18" image which appears to be the same image shown in Reyes *et al.*, Fig. 10B as a "*vWF*" Western blot.

Hence, not only is the publication by Jiang *et al.* viewed with skepticism by the scientific community, but the publication Reyes *et al.* (and by implication Furcht *et al.* U.S. Patent No. 7,015,037) is also currently under close scrutiny.

Thus, not only are the MAPCs in Jiang different from those described in Applicants' present application (and hence not anticipatory thereof), but such differences were previously made known to the Examiner.

II. The isolated cell populations of the present invention are not MSCs, MAPCs, or a mixed population of the two.

In the present office action the Examiner began by stating:

> A reconsideration of the instant application has led the Examiner to believe that *the cell population claimed are populations of MSC* (mesenchymal stem cells) *or mixed populations of MSCs and MAPCs*.

See, Paper No. 20070930, page 3, 1st paragraph (emphasis added).

With respect to the currently pending claims, and as discussed and demonstrated by the arguments, Declaration and exhibits presented herein, as well as those previously presented, it is clear that the presently claimed cell populations do not encompass MSCs, MAPCs, or mixed populations of MSCs and MAPCs, as these cells have been described in the prior art, because populations of "MSCs" and "MAPCs" do not maintain a doubling rate of less than about 30 hours after 30 cell doublings (as is required, *inter alia*, of the presently claimed cell populations).

III. Rejection of "Claim 64"

The Examiner has also asserted:

> The above discussion of *Titanium Metals* and M.P.E.P. § 2112 also applies to this ground of rejection. In this case, the claims do not require that each and every cell in the population recited in claim 64 express telomerase and have the capacity to differentiate to cells of all three lineage types; indeed the claims are drawn to an "isolated population" (*i.e.*, a population isolated from bone marrow) of cells, not to a population of isolated cells, each of which can differentiate in all three lineage types. Therefore, the claims read on the population of Haynesworth *et al.*, which Jiang *et al.* teach inherently

> comprise a few MAPCs that have the claimed properties (page 41, column 1, paragraph 3).[13]

See, Paper No. 20070930, page 7, last paragraph.

As an initial matter, there is currently no claim 64 pending in the present claims. Applicants assume, however, that the Examiner intended these comments to be applied to claim 14. If this assumption is incorrect, then clarification is respectfully requested. As a second matter, none of the claims currently pending require telomerase expression. Thus, Applicants are unclear as to the Examiner's intent with respect to commenting on telomerase expression or lack thereof. If the Examiner still deems this comment relevant in view of Applicants' present response, then clarification on this point is respectfully requested. As a third matter, Applicants also respectfully request clarification on the Examiner's perception of a substantive difference between the meaning of "an isolated population of cells" versus "a population of isolated cells" (if this comment is still deemed relevant by the Examiner in view of Applicants' present response). As a fourth matter, by way of clarification in view of the Examiner's comments, Applicants note for the record that currently pending claims 14, 21, 25 and 26 do *not* require that cells in the claimed cell populations "have the potential to differentiate into a preselected phenotype." Last, the Examiner's comments that "the claims read on the population of Haynesworth *et al.*, which Jiang *et al.* teach inherently comprise a few MAPCs that have the claimed properties (page 41, column 1, paragraph 3)." Applicants respectfully disagree. In particular, claim 14 as currently amended is drawn to:

> 14. An isolated cell population derived from human bone marrow, wherein greater than about 91% of the cells of the cell population co-express CD49c and CD90, and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings.

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[13] In Jiang, the paragraph at page 41, first column, which is cited by the Examiner reads: "We identified a rare cell within human bone marrow mesenchymal stem cell cultures that can be expanded for more than 80 population doublings. This cell differentiates not only into mesenchymal lineage cells but also endothelium[28,29] and endoderm[30]. We show that cells capable of differentiating *in vitro* to cells of the three germ layers can be selected from rodent bone marrow. These cells contribute to most somatic tissues when injected into an early blastocyst and engraft *in vivo*, where thay differentiate into tissue-specific cell types in response to cues provided by different organs."

Indeed, for the reasons provided herein and herewith, none of the publications cited (*i.e.*, neither Haynesworth, Jiang, Pittenger, Woodbury, nor Lee) can properly and legally be construed as describing, teaching, suggesting, or inherently anticipating an isolated cell population derived from human bone marrow, wherein greater than about 91% of the cells of the cell population co-express CD49c and CD90, and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings.

Thus, the presently pending claims are not anticipated by Haynesworth alone, or in any combination with Jiang, Pittenger, Woodbury, or Lee.

IV. Obviousness

In the present office action the Examiner includes allusions to a rejection based on obviousness; however, the Examiner has not specifically or explicitly alleged a rejection under 35 U.S.C. § 103. Additionally, the Examiner mixes together references to obviousness and anticipation in such manner that it is not clear to Applicants in this portion of the office action whether the Examiner is intending to render an anticipation rejection, an obviousness rejection, or both. *See*, Paper No. 09302007, page 8, second full paragraph through page 11. For example, without any reference to 35 U.S.C. § 103 the Examiner states:

> The Patent and Trademark Office is not equipped to conduct experimentation in order to determine whether or not Applicants' cells differ and, if so, to what extent, from that discussed in the references. Therefore, with the showing of the references, the burden of establishing *non-obviousness* by objective evidence is shifted to Applicants. Significantly, applicant provides no factual evidence whatsoever to refute the holding of ***anticipation*** or *obviousness*. Note specifically that on the current record the only way of overcoming such a clear holding of ***anticipation*** is factual proof that the rejection is in error. See, MPEP § 2112, disclosing that once a proper holding of ***anticipation*** is made, the burden shifts to applicant to demonstrate an *unobvious* difference between the claims and the prior art... Because applicant has not demonstrated any difference between the claimed products and the prior art products, the rejection of record clearly must be maintained... Haynesworth in light of Pittenger, Woodley [*sic*; Woodbury], Lee and Jiang (all as discussed above) [*sic*].

See, Paper No. 20070930, page 8, second full paragraph to page 9, second paragraph (emphasis added; the Examiner then recites the identical conjecture previously included in the office action.[14]). Thereafter, the Examiner repeats the above quoted paragraph. *See*, Paper No. 20070930, page 10, last paragraph through page 11.

Applicant will assume, for the sake of argument, that the Examiner intended to render both an anticipation rejection and an obviousness rejection based on Haynesworth in light of Pittenger, Woodbury, Lee and Jiang. Applicants have already, *supra*, addressed and demonstrated that the presently claimed cell populations are not anticipated by Haynesworth in light of Pittenger, Woodbury, Lee and/or Jiang. For these same reasons, the presently claimed cell populations are also not obvious in view of any combination of Haynesworth, Pittenger, Woodbury, Lee and/or Jiang.

In particular, as discussed and demonstrated above:

- Haynesworth's MSCs are CD44 negative, whereas the isolated cell populations of the present invention are CD44 positive.
- Haynesworth's MSCs are isolated from a low density, platelet-containing fraction of a 70% Percoll (low density) gradient, whereas the cell populations of present invention are isolated from a high density, mononuclear cell-containing fraction of a Histopaque 1.119® (high density) gradient.
- Isolation of Haynesworth's MSCs is described as critically reliant on one of three-types of specific cell culture media, each of which are different from the media used in isolating the cell populations of the present invention.
- Haynesworth's MSCs have significantly reduced self-renewal capacity compared to the cell populations of the present invention (*i.e.,* a mean of

[14] *See*, Paper No. 20070930, page 9, last paragraph ("It is the examiner's contention that the disclose properties [*sic*] which are not explicitly discussed in the prior art are inherent properties of the cells and cell populations disclosed in the Haynesworth reference, however if that is not the case it is apparent that the claimed characteristic of the cell population are a result of different culture conditions, or the ratio of MSCs to MAPC (as discussed in Jiang) or even the ratio of MSCs to an undetermined cell cellular 'contaminant' [*sic*] thus effecting the population as a whole.")

38 +/- 4 doublings versus at least 50 population doublings for cell populations as described in the present application).

- Haynesworth's MSCs have significantly longer and increasingly slower population doubling times compared to the presently claimed isolated cell populations, which maintain a doubling rate of less than about 30 hours after 30 cell doublings.

Hence, the presently claimed isolated cell populations are not obvious in view Haynesworth's MSCs because the disclosure in Haynesworth would not have led one of skill in the art to obtain the presently claimed isolated cell population derived from human bone marrow, wherein greater than about 91% of the cells of the cell population co-express CD49c and CD90, and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings. In fact, Haynesworth *teaches away* from the presently pending claims because, as discussed above, the MSC populations of Haynesworth are taught to have population doubling times longer than about 30 hours, and these cell populations are taught to exhibit increasingly longer population doubling times with each passage in culture.

The isolated cell populations of the presently claimed invention are also not obvious in view of Lee. As discussed above:

- Lee describes a population of MSCs which are CD13 *negative*. *See*, Lee, page 1278, right column. In contrast, the cell populations of the present invention are CD13 *positive*. *See*, Second Kopen Declaration and **Exhibit D**.
- Lee's MSCs have longer doubling times than the presently claimed cell populations. Lee states "The doubling time of these BM-derived cells was found to be between 40 to 52 hours (data not shown)." *See*, Lee at page 1278, right column, 1st paragraph. In contrast, the cell populations of the presently claimed invention maintain a doubling rate of less than about 30 hours after 30 cell doublings.

Hence, given that the disclosures of Haynesworth and Lee describe distinctly different cell populations from the isolated cell populations of the present invention, these publications (together or alone) do not render obvious the presently claimed isolated cell population derived from human bone marrow, wherein greater than about 91% of the cells of the cell population co-express CD49c and CD90, and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings.

The isolated cell populations of the presently claimed invention are also not obvious in view of Woodbury. As discussed above, the human MSCs described in Woodbury are the same as those described in Azizi *et al.* However, the MSCs of Azizi are not the same population of cells disclosed in Applicants present specification. For example, (as discussed herein and herewith):

- The human MSCs of Azizi require cell culture supplementation with PDGF-AA for optimal growth. In contrast, Applicants cell populations do not require growth factor supplementation (as discussed in the May 2007 Declaration).
- The MSCs of Azizi have a population doubling time (PD) of about, at best, approximately 96 hours *with* PDGF-AA supplementation. In contrast, the cell populations or the presently claimed invention maintain a doubling rate of less than about 30 hours after 30 cell doublings.

Hence, given the distinctly different cell populations described in Woodbury, the disclosures of Haynesworth and Woodbury (together or alone) would not render inherently obvious the presently claimed isolated cell population derived from human bone marrow, wherein greater than about 91% of the cells of the cell population co-express CD49c and CD90, and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings.

The isolated cell populations of the presently claimed invention are also not obvious in view of Pittenger. As discussed above, Pittenger describes a population of MSCs which are CD106 and CD62L *positive*. In contrast, the isolated cell populations of the present invention are CD106 and CD62L *negative* (as discussed in the Second Kopen

Declaration). Therefore, given the distinctly different cell populations described in Pittenger, the disclosures of Haynesworth and Pittenger (together or alone) would not render obvious the presently claimed isolated cell population derived from human bone marrow, wherein greater than about 91% of the cells of the cell population co-express CD49c and CD90, and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings.

The isolated cell populations of the presently claimed invention are also not obvious in view of Jiang. As discussed above, the *mouse* cells described in Jiang are CD44 and MHC Class I negative. In contrast, Applicants previously demonstrated that the isolated cell populations of the present invention are CD44 and MHC Class I positive. *See*, May 2007 Declaration. Hence, given the distinctly different cell populations described in Jiang, the disclosures of Haynesworth and Jiang (together or alone) would not render obvious the presently claimed isolated cell population derived from *human* bone marrow, wherein greater than about 91% of the cells of the cell population co-express CD49c and CD90, and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings.

In sum, as requested by the Examiner in the presently pending office action, Applicants have provided factual evidence to refute holdings of anticipation and obviousness based on Haynesworth, Pittenger, Woodbury, Lee and Jiang.

V. Ranges

The Examiner has also conjectured:

> It would appear that any actual differences between the cell populations of Haynesworth and the claimed cell populations would be a question of the concentration of cells or the purity of the population. As such even if the claimed populations are not anticipated by the Haynesworth populations, generally, differences in concentration will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical.

See, Paper No. 20070930, page 9, last paragraph to page 10, first paragraph. The Examiner then cites selections of case law pertaining to patentability based on particularly claimed numerical ranges (*i.e.*, *In Re Aller*, *In Re Peterson*, and *In Re Hoeschele*).

Applicants respectfully disagree and traverse the rejection. As an initial matter, Applicants note that the grounds of this rejection are again based on the Examiner's speculation and conjecture that the presently claimed cell populations *might possibly* have been present, even though unidentified, as some proportion of a cell population described in Haynesworth. As discussed at length above, however, Applicants have already demonstrated that Haynesworth isolated a different population of cells compared to those of the present invention. Furthermore, Haynesworth does not describe *any range* of cell populations wherein the cells of the cell population co-express CD49c and CD90, and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings. Hence, anticipation and obviousness rejections based on a "question of the concentration of the cells or the purity of the population" is legally untenable and improper because Haynesworth does not describe or suggest *any concentration* ***or*** *any proportion* of cells having the properties of the presently claimed cell populations. In fact, Haynesworth *teaches away* from the presently pending claims because, as discussed above, the MSCs of Haynesworth are taught to have population doubling times longer than about 30 hours, and the MSCs of Haynesworth are taught to exhibit increasingly longer population doubling times with each passage in culture.

Likewise, the case law cited by the Examiner with respect to patentability based on claimed ranges is inapplicable in the present case because the prior art does not identify any range of the presently claimed cell populations.

For example, the Examiner alleges support from *In Re Aller* by quoting "*[W]here the general conditions of a claim are disclosed in the prior art*, it is not inventive to discover the optimum or workable ranges by routine experimentation." *See*, Paper No. 20070930, page 10, first paragraph (emphasis added). Applicants note that this instruction is inapplicable in the present case because the cited prior art does not disclose "the general conditions" of *any* cell population derived from human bone marrow, wherein the cells of the cell population co-express CD49c and CD90, and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings.

Similarly, the Examiner alleges support from *In Re Peterson* by quoting, "The normal desire of scientists or artisans *to improve upon what is already generally known*

provides the motivation to determine *where in a disclosed set of percentage ranges* is the optimum combination of percentages". *Id.* (emphasis added). Again, Applicants submit that this instruction is inapplicable in the present case because the presently pending claims are not drawn to subject matter which was already "generally known" nor does the cited prior art disclose *any* "set of percentage ranges" with respect to a cell population derived from human bone marrow, wherein the cells of the cell population co-express CD49c and CD90, and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings.

Likewise, the Examiner alleges support from *In Re Hoeschele* by quoting "Claimed elastomeric polyurethanes which fell within the broad scope of the references were held to be unpatentable thereover because, among other reasons, there was no evidence of the criticality of the *claimed ranges of molecular weight or molar proportions*." *Id.* Applicants submit that this quote is inapplicable in the present case because the cited prior art does not disclose *any range* of cell population derived from human bone marrow, wherein the cells of the cell population co-express CD49c and CD90, and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings.

In sum, Applicants have provided factual evidence to refute rejections based anticipation and obviousness in view of Haynesworth, Pittenger, Woodbury, Lee and Jiang. Therefore, it is respectfully requested that all rejections of pending claims 14, 21, 25 and 26 be reconsidered and withdrawn.

VI. Double Patenting Rejection

The Examiner has also provisionally rejected claims 14, 19-21 and 25-26 on the alleged grounds of nonstatutory obviousness-type double patenting as being "unpatentable over claims 14, 19-21 and 24-25 of copending Application No. 10/251685." *See*, Paper No. 20070930, page 12 through page 14. The Examiner notes that this is a "*provisional* obviousness-type double patenting rejection because the conflicting claims have not in fact been patented." *Id.* (emphasis in original).

As an initial matter, Applicants note that claims 1-11 and 57-60 are currently pending in U.S. Application No. 10/251,685 (with claims 50-56 & 61-62 previously

canceled and claims 12-49 previously withdrawn from consideration by the Examiner). However, Applicants respectfully request that the currently asserted double patenting rejection be held in abeyance until claimed subject matter is otherwise deemed allowable, at which time Applicants will consider filing a terminal disclaimer in compliance with 37 C.F.R. §§ 1.321(c) or 1.321 (d) in the later filed Application No. 10/251,685 if necessary to overcome an obviousness-type double patenting rejection.

VII. Limits on differentiation capacity of isolated cell populations of the invention

Applicants would like to provide notice for the record that they have recently discovered that the isolated cell populations of the present invention appear to lose osteogenic differentiation capacity with continued passage *in vitro* at about 25-30 population doublings. In particular, it has been discovered that after about 25-30 population doublings the isolated cell populations of the invention have lost the ability to diffentiate into bone cells as demonstrated, for example, by loss of ability to generate calcium deposits under optimal osteoinductive conditions (*i.e.*, culture conditions which typically induce a phenotypic marker, or markers, of bone differentiation).

For example, it has now been observed that ABM-SC at about 16 population doublings exhibit a calcium deposition increase of approximately 6-fold when osteoinductive media (OIM) is supplemented with Noggin (*i.e.*, ABM-SC at about 16 population doublings deposited ~5 micrograms calcium/cell culture well under OIM conditions and ~30 micrograms/well under OIM + Noggin conditions). Moreover, although ABM-SC lost the capacity to deposit detectable levels of calcium beyond about 16 population doublings under standard OIM conditions, this could be reversed by supplementing OIM with Noggin (*i.e.*, ABM-SC at about 25 population doublings deposited no detectable calcium under OIM conditions whereas these same cells deposited ~5 micrograms calcium/well under OIM + Noggin conditions). In contrast, beyond about 30 population doublings (*e.g.*, at about 35 and 43 populations doublings) exABM-SC did

not deposit detectable levels of calcium under OIM or OIM + Noggin (optimal) conditions.

Despite this apparent loss of at least some differentiation capacity, expression of the phenotypic cell surface markers, as demonstrated by the data presented herein, continue to remain constant even after 25-30 population doublings. The isolated cell populations continue to maintain consistent population doubling rates of less than about 30 hours. And, the cells continue to secrete therapeutically useful compositions after 25-30 population doublings. Thus, the therapeutic utility of the presently claimed cell populations is maintained even after more than about 25-30 population doublings.

Applicants would also like to clarify for the record statements presented in a Reply to an Office Action submitted by Applicants on Dec. 22, 2003. In particular, on page 16 of the Reply it was stated "The isolated cell populations of Applicants' claimed invention, as amended, *can* differentiate into a pre-selected phenotype including a chondrocyte, an astrocyte, an oligodendrocyte, a neuron, an osteoclast, an osteocyte, an osteoblast, a cardiomyocyte, a pancreatic islet cell, a skeletal muscle cell, a smooth muscle cell, a hepatocyte and a retinal ganglial cell." (Emphasis added). Similarly, on page 17 of this same reply it was stated, "Applicants' claimed cell population *can* differentiate into a pre-selected phenotype following treatment of a mammal." (Emphasis added).

In view of these statements, Applicants would like to clarify for the record that it has now been discovered and determined that the isolated cell populations of the present invention lose at least some differentiation capacity after about 25-30 population doublings *in vitro*. It has also been discovered and determined that the isolated cell populations of the present invention do *not* appear to "differentiate into a pre-selected phenotype following treatment of a mammal." This lack of differentiation into a pre-selected phenotype following treatment of a mammal, however, is therapeutically beneficial because the cells of the present invention do not differentiate into unwanted tissue types such as bone (as do some "stem" cells) when injected into, for example, cardiac tissue.

Applicants would also like to note for the record that these more recent discoveries do not affect the teachings of the specification as originally filed because: 1) early passage

cells retain differentiation capacities, and 2) the specification describes the isolated cells of the present invention as *having the potential* for multipotent differentiation, but it does not assert that the cell populations of the invention *always* possess this capacity. For example, the specification states:

> Advantages of the cell based therapies of the claimed invention include, for example, incorporation of the cells into the tissue...the incorporated cells *have the potential* to differentiate or develop into neuronal, glial or other cells...

See, specification, page 5, lines 22-26.

> The substantially homogenous cell population which co-express CD49c and CD90 *can have the potential* to differentiate into a preselected phenotype (e.g., chondrocytes, astrocytes, oligodendrocytes, neurons, bone, osteoclasts, osteoblasts, cardiomyocytes, pancreatic islet cells, skeletal muscle, smooth muscle, hepatocytes and retinal ganglial cells). The potential to differentiate into a preselected phenotype refers to the ability of the cell population to change to a functional cell type.

See, specification, page 8, lines 13-18 (emphasis added).

> The cell population which co-express CD49c, CD90 and telomerase *has the potential* to differentiate into a preselected phenotypes (e.g., a chondrocyte, an astrocyte, an oligodendrocyte, a neuron, osteocyte, osteoblast, osteoclast, a cardiomyocyte, a pancreatic islet cell, a skeletal muscle, a smooth muscle, a hepatocyte and a retinal ganglial cell).

See, specification, page 13, line 28 to page 14, line 3 (emphasis added).

> The transplantation of the substantially homogenous cell population of the invention into a patient suffering from a neurological condition *may result* in the differentiation of the cells of the invention into cells which normally function in the nervous tissue affected in the human with the neurological condition thereby treating a myriad of neurological conditions including, for example, Parkinson's disease, ALS, spinal cord injury, brain tumors, stroke.

See, specification, page 19, lines 10-15 (emphasis added).

> The cell populations of the invention *may have the capacity* to respond to intrinsic signals (e.g., at the sites of transplantation or when incorporated into tissues and organs) and exogenous cues *to differentiate* into numerous cell types (e.g., neuronal, glial, astrocytes, oligodendrocystes) in the human.

See, specification, page 19, lines 18-21 (emphasis added).

Hence, prior to approximately 25-30 population doublings the isolated cell populations of the present invention retain at least some osteogenic differentiation capacity. Further, the isolated cell populations of the present invention continue to secrete therapeutically useful compositions after 25-30 population doublings. Therefore, the therapeutic utility of the presently claimed cell populations is not only maintained after more than about 25-30 population doublings, but is actually considered by Applicants to be enhanced because, upon introduction into a host organism, there is a reduced risk of the cells differentiating into an unwanted tissue type (for example, differentiating into bone when injected into or near heart tissue).

VIII. Comments on the Examination of the Present Application

With all due respect, Applicants are concerned about the unacceptable manner in which prosecution of the present application has continued to drag on in the Patent and Trademark Office. Time and again the Applicants have been able to distinguish their invention from the prior art, yet the Examiner has continued to issue rejections on repetitive and increasingly questionable grounds. In the present office action, the prior art cited comprises a cumulative second round of citations referencing the same populations of bone marrow-derived cells over which Applicants have already distinguished their claims in responses to previous office actions. Furthermore, all of the prior art cited in the present office action was submitted and considered by the Examiner several years ago![15]

[15] Haynesworth *et al.*, U.S. Patent 5,733,542 was previously cited to the Examiner as reference "AF2" in Applicants' Second Supplemental Information Disclosure Statement submitted on May 17, 2002. Furthermore, this reference was signed as *considered by the Examiner on July 16, 2003*. Pittenger *et al., Science 284*:143-147 (1999) and Woodbury *et al., J. Neurosci. Res. 61*: 364-370 (2000) were previously cited to the Examiner as references "AZ2" and "AX3," respectively, in an IDS submitted to the Patent Office on Jan. 22, 2002 and again submitted to the Patent Office on Dec. 22, 2003. *See*, "Transmittal of Previously Filed Information Disclosure Statement and Previously Cited References," submitted by Applicants on Dec. 22, 2003. Furthermore, these references were signed as *considered by the Examiner on Mar. 15, 2004*. Lee *et al., Hepatology 40*: 1275-1284 (2004) and Jiang *et al.*, Nature 418:41-49 (July 2002) are not prior art against the present application under 35 U.S.C. § 102 (b).

Accordingly, the present application has now been subject to:

- Four rounds of non-final rejections and responses;
- Two rounds of final rejections and responses;
- One Request for Continued Examination;
- One Notice of Allowance;
- One Issue Fee Payment;
- A Withdrawal from Issue;
- A lengthy suspension of prosecution due to a potential interference that was never declared (instead a patent with questionable data (as discussed *supra*) was granted to Furcht *et al.*); and,
- Three interviews with the Examiner.

Applicants respectfully submit that the presently pending claims are in condition for prompt and immediate allowance. Applicants urge the Examiner to finally pass this case to the issuance it rightfully deserves.

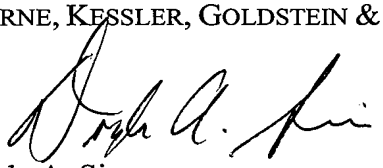
Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



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2560.0020000_Response_to_OA_10-05-2007_FINAL_03-04-08.doc

EXHIBIT B

SIGMA-ALDRICH®

LEUKOCYTE SEPARATION

(Procedure No. 1119)

INTENDED USE

A method using HISTOPAQUE®-1119 and HISTOPAQUE®-1077 for separating mononuclear cells and granulocytes. HISTOPAQUE®-1119 reagents are for "In Vitro Diagnostic Use".

In 1968, Boyum[1] described gradient density centrifugation methods for isolation of mononuclear cells from circulating blood and bone marrow. Solutions used for this purpose consist of a polysucrose and a radiopaque medium. HISTOPAQUE®-1077 is such a solution adjusted to a density of 1.077. When blood is layered onto HISTOPAQUE®-1077 and subjected to centrifugal forces, mononuclear cells are held at plasma HISTOPAQUE® interphase while erythrocytes and granulocytes gravitate to the bottom. It would appear feasible to devise a system whereby cells of the myeloid series could also be harvested employing a one-step procedure. HISTOPAQUE®-1119 was developed to achieve this purpose and is based on observations by English and Andersen.[2]

According to the Sigma-Aldrich procedure, a double gradient is formed by layering an equal volume of HISTOPAQUE®-1077 over HISTOPAQUE®-1119. Whole blood is carefully layered onto the upper HISTOPAQUE®-1077 medium. The tubes are then centrifuged at 700 x g for 30 minutes. Cells of the granulocytic series are found at the 1077/1119 interphase whereas lymphocytes, other mononuclear cells and platelets are found at the plasma/1077 interphase.

REAGENT

HISTOPAQUE®-1119, Catalog No. 1119-1
Polysucrose, 6.0 g/dl and sodium diatrizoate, 16.7 g/dl. Aseptically filtered.

STORAGE AND STABILITY:
Store HISTOPAQUE®-1119 in refrigerator (2–8°C). Protect from light. Reagent label bears expiration date.

DETERIORATION:
A cloudy appearance indicates deterioration of the product.

PREPARATION:
HISTOPAQUE®-1119 is ready for use. Warm to 18–26°C before use.

PRECAUTIONS:
Normal precautions exercised in handling laboratory reagents should be followed. Upon contact with human source substances, treat all reagents and equipment as potentially biohazardous. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

US Risks and Safety Statements
HISTOPAQUE® solutions 1077-1 and 1119-1 are HARMFUL. May cause sensitization by inhalation and skin contact. Wear suitable protective clothing. Target organ: Blood.

EU Risks and Safety Statements
HISTOPAQUE® solutions 1077-1 and 1119-1 are HARMFUL. May cause sensitization by inhalation and skin contact. Do not breathe vapor. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

PROCEDURE

SPECIMEN COLLECTION:
It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

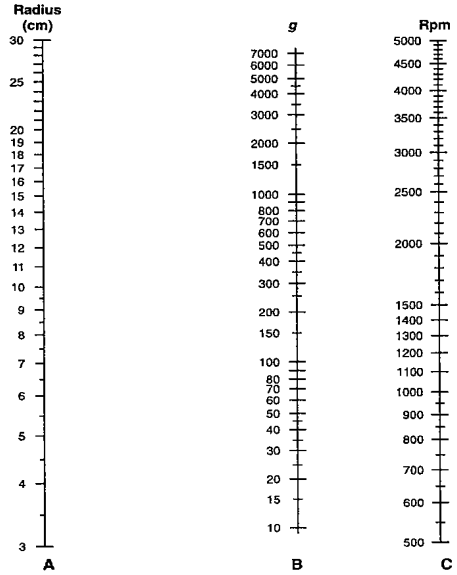
Collect 6 ml venous blood in preservative-free heparin or EDTA.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:
HISTOPAQUE®-1077, Catalog No. 1077-1, Polysucrose, 5.7 g/dl, and sodium diatrizoate, 9.0 g/dl. Aseptically filtered.

Centrifuge (Swinging Bucket Rotor) capable of generating 700 x g
Centrifuge tubes, 15 ml plastic, conical

NOTES:
1. 50 ml centrifuge tubes may be used. Alter stated procedure by using 12 ml of HISTOPAQUE®-1077, 12 ml of HISTOPAQUE®-1119, and 24 ml of whole or diluted blood.
2. On occasion it may be necessary to dilute blood 1:2 or 1:4, depending upon absolute cell numbers. The possibility of overloading the gradient exists.
3. Avoid use of powdered gloves. Glove powder will activate monocytes and cause lower yields.
4. Avoid use of high binding plastics such as polystyrene. Polystyrene may bind cells to the centrifuge tube walls.
5. Prepare gradient immediately before use. Preparing gradients in advance will allow diffusion to occur and result in poor cell recovery.
6. Underlaying the HISTOPAQUE®-1119 will also produce an acceptable gradient.
7. Other anti-coagulants may be used; however the choice of anti-coagulant may affect cell recovery. As blood ages the cell recoveries will drop.
8. The procedure section of this insert employs use of isotonic phosphate buffered saline as a diluent and washing fluid. Other reagents such as cell medium RPMI 1640 supplemented with fetal bovine serum may be used.
9. The procedure described is for isolation of cells from 6 ml of whole blood. Volumes may be increased or decreased as necessary.
10. The use of a "normal" patient is recommended as a control for each run.

NOMOGRAM FOR DETERMINING RELATIVE CENTRIFUGAL FORCES:

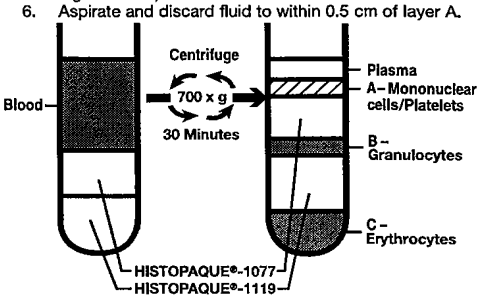


A nomogram is used to derive the rpm setting for your centrifuge.

How to establish the rpm required to obtain 700 x g for Procedure No. 1119.
1. Measure the radius (cm) from the center of the centrifuge spindle to the end of the test tube carrier. Mark this value on scale A.
2. Mark the relative centrifugal force (e.g., 700) on scale B.
3. With a ruler, draw a straight line between points on columns A and B, extending it to intersect column C. The reading on column C is the rpm setting for the centrifuge.

PROCEDURE:
1. Add 3 ml HISTOPAQUE®-1119, to a 15 ml conical centrifuge tube.
2. Carefully layer 3 ml of HISTOPAQUE®-1077, onto the HISTOPAQUE®-1119.
3. Carefully layer 6 ml of whole blood onto the upper gradient of the tube from Step 2.
4. Centrifuge at 700 x g for 30 minutes at room temperature (18–26°C). Centrifugation at lower temperatures, such as 4°C, may result in cell clumping and poor recovery.

NOTE: The rpm required to generate 700 x g can be calculated using the nomogram in this insert.
5. Carefully remove centrifuge tubes. Two distinct opaque layers should be observed (layers A and B in Figure below).
6. Aspirate and discard fluid to within 0.5 cm of layer A.



Transfer cells from this layer to a tube marked "mononuclear".
7. Aspirate and discard remaining fluid to within 0.5 cm of layer B. Transfer cells from this layer to a tube labeled "granulocytes".
8. Wash the cells by addition of 10 ml isotonic phosphate buffered saline to the tubes. Centrifuge 10 minutes at 200 x g. Remove the supernatant and discard.
9. Resuspend the cells by gentle aspiration with a Pasteur pipet.
10. Repeat Steps 8 and 9 two times.
11. Resuspend cells in an appropriate volume of isotonic phosphate buffered saline.

At this point a variety of assays can be performed. The procedures are chosen according to individual discretion.

PERFORMANCE CHARACTERISTICS

Erythrocytes should pellet to the bottom of the centrifuge tube. Granulocytes should band at the interface between the HISTOPAQUE®-1119 and the HISTOPAQUE®-1077. Mononuclear cells should band at the interface between the HISTOPAQUE®-1077 and the plasma

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES

1. Boyum A: Separation of leukocytes from blood and bone marrow. Scand J Clin Lab Invest 21: suppl 97:77, 1968
2. English D, Andersen BR: Single-step separation of red blood cells. Granulocytes and mononuclear leukocytes on discontinuous density gradient of ficoll-hypaque. J Immunol Methods 5:249, 1974

Procedure No. 1119
Previous Revision: 2003-04
Revised: 2003-09

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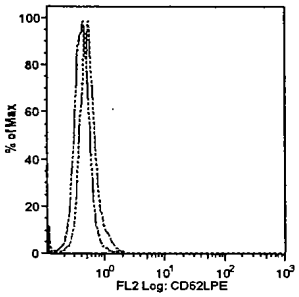
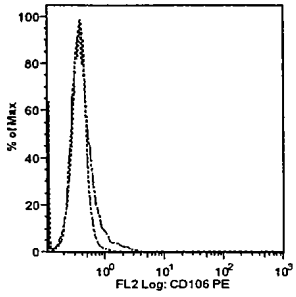
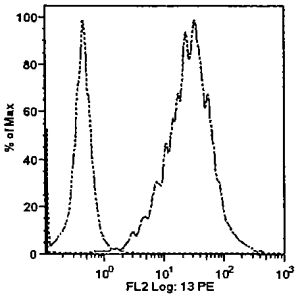
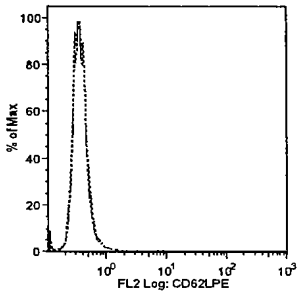
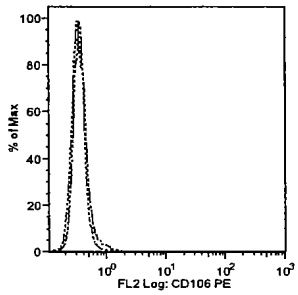
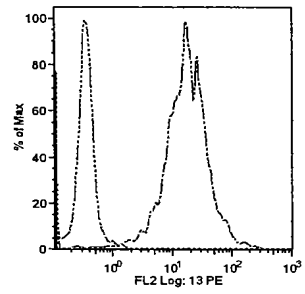
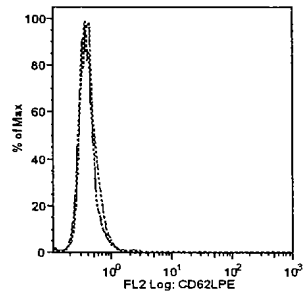
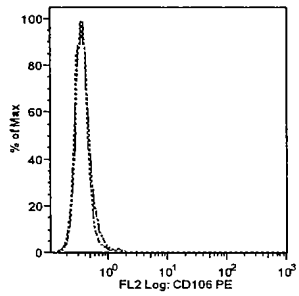
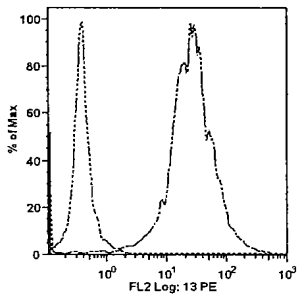
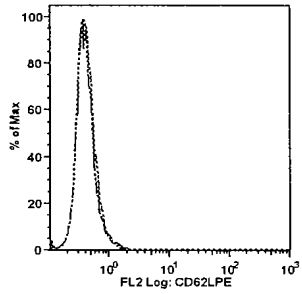
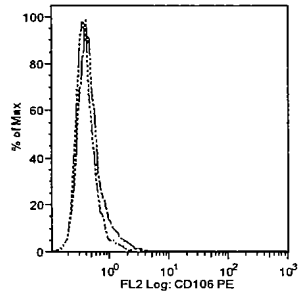
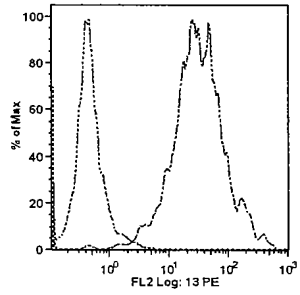
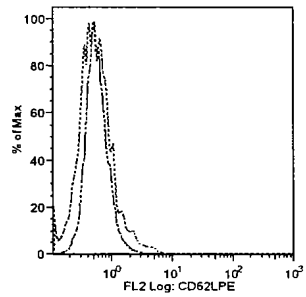
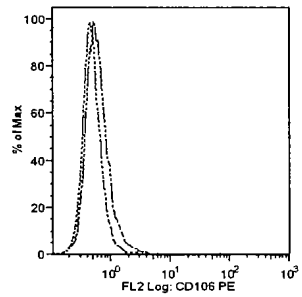
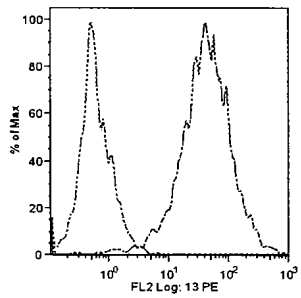
Exhibit C

Component	09/960,244 Medium	Haynesworth Media		
	Alpha MEM (mg/L)	DMEM (mg/L)	BGJB Medium (mg/L)	Ham F12 (mg/L)
$CaCl_2$ $2H_20$				44
$CaCl_2$ (anhydrous)	200.0	200.0		
$CuSO_4$ $5H_20$				0.00249
$FeSO_4$ $7H_20$				0.834
KCl	400.0	400.0	400.00	223.6
MgCl2 6H20				122
$MgSO_4$ (anhydrous)	97.7	97.7	200.00	
KH_2PO4			160.00	
NaCl	6800.0	6400.0	5300.00	7599
Calcium Lactate			550.00	
$NaH_2PO_4 \cdot H_2O$	140.0	125.0	90.00	268
$ZnSO_4$ $7H_20$				0.863
L-Alanine	25.0		250.00	8.9
L-Arginine HCl	127.0	84.0	175.00	211
L-Asparagine H_2O	50.0			15.01
L-Aspartic Acid	30.0		150.00	13.3
L-Cysteine $HCl \cdot H_2O$	100.0		101.00	35.12
L-Cystine 2HCl	31.3	662.6		
L-Glutamic Acid	75.0			14.7
L-Glutamine	292.0	584.0	200.00	146
Glycine	50.0	30.0	800.00	7.5
L-Histidine $HCl \cdot H_2O$	41.9	42.0	150.00	20.96
L-Isoleucine	52.5	105.0	30.00	3.94
L-Leucine	52.4	105.0	50.00	13.1
L-Lysine HCl	72.5	146.0	240.00	13.5
L-Methionine	15.0	30.0	50.00	4.48
L-Phenylalanine	32.0	66.0	50.00	4.96
L-Proline	40.0		400.00	34.5
L-Serine	25.0	42.0	200.00	10.5
L-Threonine	48.0	95.0	75.00	11.9
L-Tryptophan	10.0	16.0	40.00	2.04
L-Tryosine $2Na \cdot 2H_2O$	51.9	103.8	40.00	5.4
DL-Valine			65.00	
L-Valine	46.0	94.0		11.7
L-Ascorbic Acid	50.0		50.00	
d-Biotin	0.1		0.20	
D-Ca Pantothenate	1.0	4.0		
Choline Chloride	1.0	4.0	50.00	
Folic Acid	1.0	4.0	0.20	
Myo-Inositol	2.0	7.2	0.20	
Nicotinamide			20.00	
Niacinamide	1.0	4.0		
para-aminobenzoic acid			2.00	
Pyridoxal HCl	1.0	4.0	0.20	

Riboflavin	0.1	0.4	0.20	
Thiamine HCl	1.0	4.0	4.00	
Vitamin B-12	1.4		0.04	
D-Glucose	1000.0	1000.0	101000.00	1802
Lipioc Acid	0.2			0.21
Phenol Red (Sodium)	11.0	15.0	20.00	1.2
Sodium Pyruvate	110.0	110.0		110
$NaHCO_3$	2200.0	3700.0		1176
Sodium Acetate			50.00	
α-tocopherol phosphate (disodium salt)			1.00	
Thymidine				0.73
Putrescine 2HCl				0.161
Linoleic Acid				0.084
Hypoxanthine				4.1

Exhibit D

	anti-**CD62L** (blue) vs neg. control ab. (red)	anti-**CD106** (blue) vs neg. control ab. (red)	anti-**CD13** (blue) vs neg. control ab. (red)
18.8 PD			
27.2 PD			
35.2 PD			
43.9 PD			
51.7 PD			

CD49c/CD90 FACS Analysis of Cells Isolated Via Ammonium Chloride Lysis or HISTOPAQUE® 1.119 Gradient Separation Procedures

FACS analysis was performed on aspirates of human adult bone marrow-derived cells prepared as described in Example 1 (red blood cell/ammonium chloride lysis procedure) and Example 2 (density gradient separation procedure) of U.S. Patent Application 09/960,244.

Resuspended cells (approximately 10^6) were aliquoted into12x75mm Flow Cytometry tubes and repelleted at 500xg for 5 minutes. The HBSS was removed and 25 microliters of the following antibodies (all obtained from Becton Dickenson), alone or in combination, were placed into each tube: mouse IgG1k-FITC or -PE (clone MOPC-21) CD49c-PE (cl. C3II.1), CD90-FITC (cl. 5E10). Tubes were gently vortexed and incubated for 30 minutes at 4°C. Cells were then washed in HBSS/1% bovine serum albumin, centrifuged (30 min, 4°C) and the resulting cellular pellet fixed by the addition of 250 microliters of 2% paraformaldhyde/HBSS. Flow cytometric analysis was performed employing a Becton-Dickenson FACSVantage SE cytometer and analyzed using CellQuest® software. Depicted results represent data collected from 2,500- 10,000 events per panel.

After compensation for non-specific antibody staining using mouse IgG1 isotype controls, cellular expression of CD49c and CD90 in the bone marrow cells was assessed. As seen in Panel A, after red blood cell/ammonium chloride lysis only, approximately 0.05% of recovered cells co-express CD49c and CD90 (upper right quadrant). As seen in Panel B, after density gradient separation using HISTOPAQUE® 1.119, the fraction of cells co-expressing CD49c and CD90 was approximately 10 fold higher at 0.51% than the concentration obtained via the red blood cell/ammonium chloride lysis procedure.

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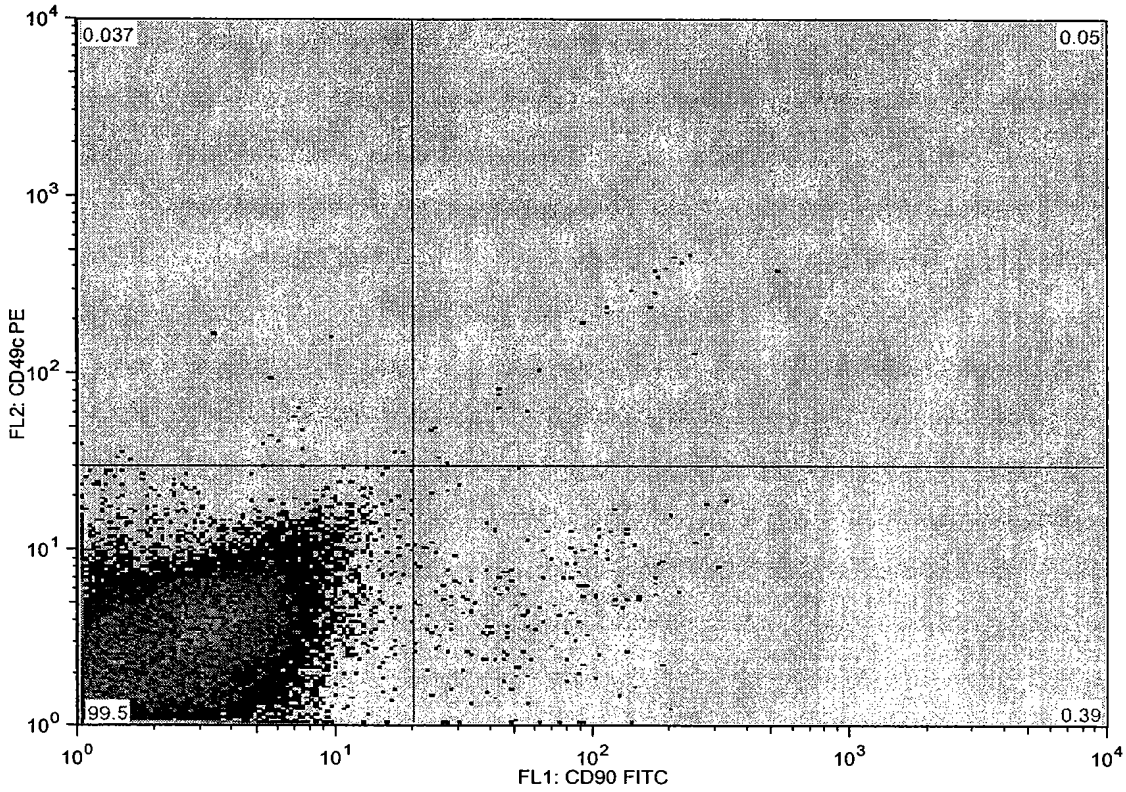

Panel A

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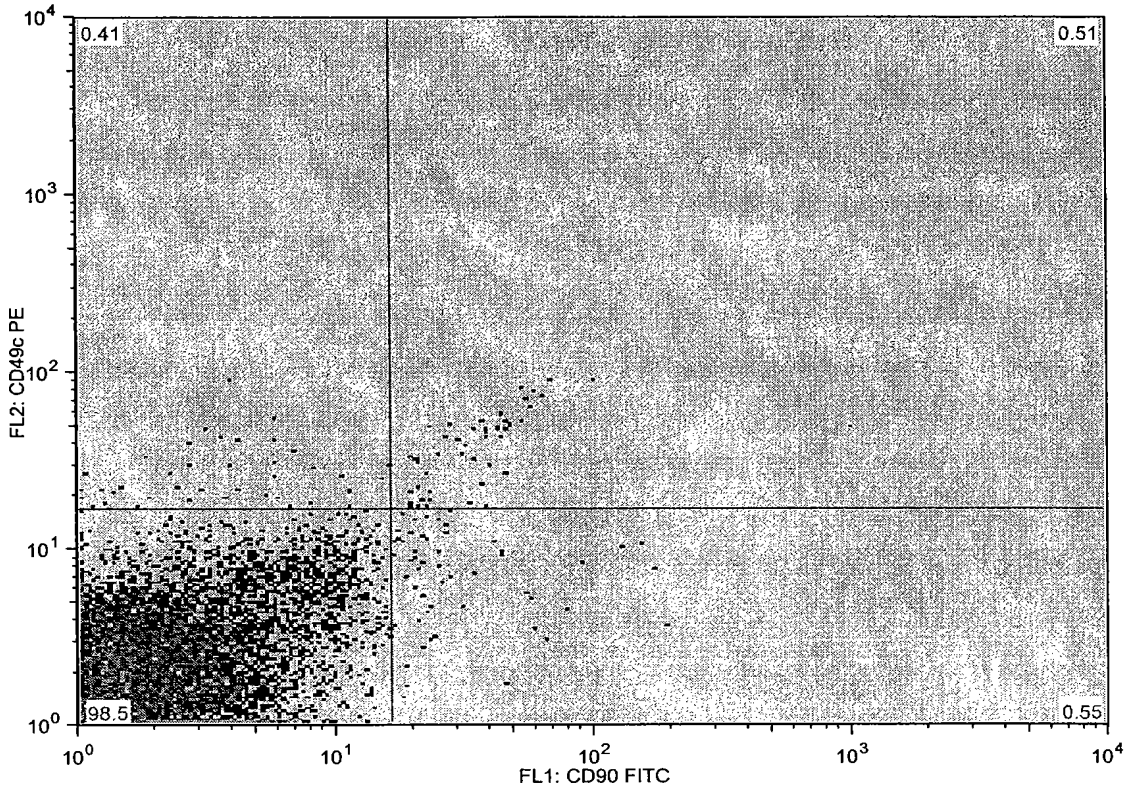

Panel B